AMENDMENTS TO THE SPECIFICATION

On page 1, line 3, please delete: -- 60/xxx,xxx --,

and insert: -- -- 60/248,275, filed on November 14, 2000 -- --.

The amendment adds no new matter or claim of priority.

On page 22, following: -- (intravascular delivery of plasmid DNA). -- on line 2,

please add: -- --

Figure 3. Western blot detection of luciferase expression in 293 cells using antibodies from serum of genetically immunized mice receiving 3 injections (prime + 2 boosts).

Figure 4. Western blot detection of luciferase expression in 293 cells using antibodies from serum of genetically immunized mice receiving 4 injections (prime + 3 boosts). -- --

The descriptions are taken from the text in Example 8. The brief descriptions of figures 3 and 4 add no new matter.

A Replacement Sheet showing the changes made is included.

METHODS FOR GENETIC IMMUNIZATION

This application claims priority benefit of U.S. Provisional Application Serial No. ~~60/xxx,xxx~~ 60/248,275, filed on November 14, 2000.

FIELD OF THE INVENTION

The present invention relates to compositions and methods for transferring nucleic acids into cells *in vivo* for the purpose of eliciting an immune response. In preferred embodiments, the compositions comprise intravascular delivery systems providing high transfection efficiency. In additional preferred embodiments, the compositions comprise delivery systems providing nucleic acid transfer complexes that transfect cells with high efficiency. In additional preferred embodiments, methods for detection of an immune response following genetic immunization are disclosed.

BACKGROUND OF THE INVENTION

Genetic vaccines

The development of vaccines is frequently heralded as one of the most important medical breakthroughs. Prevention of disease has provided increases in human life expectancy, lowered healthcare costs, and has resulted in enhanced quality of life. Yet more widespread use is hampered by three problems. First, it remains difficult to create effective vaccines for new microbes. Second, distribution and administration of current vaccines is expensive (requiring cooling and injection equipment). Third, parental vaccine delivery is not well accepted (discomfort with the use of needles and reactivity to adjuvant resulting in poor compliance). Genetic (or DNA) vaccines can extend the array of vaccines, and overcome these hurdles. With a classic vaccine, the antigen itself is introduced - either in the form of attenuated, killed or inactivated microbe, or as purified (recombinant) protein. With a genetic vaccine, the coding sequence for the antigen (or part of the antigen) is introduced. Following transfection of a host cell, the antigen is produced *in situ*.

Genetic vaccinations potentially overcome the three major hurdles listed above. By expressing antigens *in vivo* (e.g., after intramuscular injection of plasmid DNA expressing the antigen), one avoids the use of killed or attenuated microbes. Also, it is now possible to create vaccines for peptides that previously could not be produced or isolated. Since the full cellular biochemical machinery is available, antigens that are heavily modified can be used efficiently. A major result is the induction of strong CTL responses, where conventional subunit vaccines are skewed toward humoral responses. Since each

membranes were incubated with a 1;2,000 dilution of serum collected from a pCI-Luc immunized mouse (intravascular delivery of plasmid DNA).

Figure 3. Western blot detection of luciferase expression in 293 cells using antibodies from serum of genetically immunized mice receiving 3 injections (prime + 2 boosts).

Figure 4. Western blot detection of luciferase expression in 293 cells using antibodies from serum of genetically immunized mice receiving 4 injections (prime + 3 boosts).

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

The term "nucleic acid" is a term of art that refers to a polymer containing at least two nucleotides. "Nucleotides" contain a sugar deoxyribose (DNA) or ribose (RNA), a base, and a phosphate group. Nucleotides are the monomeric units of nucleic acid polymers. Nucleotides are linked together through the phosphate groups to form nucleic acid. A "polynucleotide" is distinguished here from an "oligonucleotide" by containing more than 100 monomeric units; oligonucleotides contain from 2 to 100 nucleotides. "Bases" include purines and pyrimidines, which further include natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and other natural analogs, and synthetic derivatives of purines and pyrimidines, which include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides. The term nucleic acid includes deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA"). The term nucleic acid encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.